Citation:

Kelley DS, Rasooly R, Jacob RA, Kader AA, Mackey BE. Consumption of Bing sweet cherries lowers circulating concentrations of inflammation markers in healthy men and women. *J Nutr.* 2006 Apr;136(4):981-6.

PubMed ID: 16549461

Study Design:

Time Series Study

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To determine the effects of consuming sweet cherries on plasma lipids and markers of inflammation in healthy men and women.

Inclusion Criteria:

- Found to be in "good health" based on screening
- Adult (18 or over)
- Informed written consent

Exclusion Criteria:

- In "poor health"
- Obese (BMI > 30 kg/m^2)
- Using nutritional supplements, medications, alcohol, or recreational drugs on a regular basis

Description of Study Protocol:

Recruitment

Candidates were recruited from the Davis, CA area and screened for good health by a medical history questionnaire, physical exam, and standardized blood tests including a complete blood cell (CBC) count with leukocyte differential, clinical chemistry panel, lipid panel, and tests for infectious disease.

Design: Time Series Study

A single group completed a 64-day study with 3 metabolic periods:

- Baseline period of 8 days (d 0-7)
- Cherry intervention period of 28 days (d 8-35)
- Postintervention period of 28 days (d 36-64)

Blinding used (if applicable): not applicable

Intervention (if applicable)

- The intervention lasted from June - August 2003 during California's fresh cherry season.
- Subjects were asked to not change their activity level and diet except to replace an equivalent amount of dietary carbohydrate with carbohydrates from cherries (300 g, equivalent to 280 g when pitted at the time of consumption; ~216 kcal, ~11% daily energy intake) during the 28 days of cherry consumption.
- They were instructed to limit the consumption of foods rich in polyphenols (berries, tea, wine, grape products, fruit juice, apples, and broccoli) throughout the study.
- Bing cherries were sorted to discard cracked, decayed, and unripe fruit and weighed into aliquots of ~45 cherries each, placed in bags, and kept at 0 degrees Celsius until distribution.

Statistical Analysis

- Repeated measures model with a first-order autoregressive covariance structure among the repeated measures.
- The fixed effect was day and the random effect was subject.
- One-tailed tests for single df contrasts were used to compare the treatment periods.

Data Collection Summary:

Timing of Measurements

- Dietary records (24 hour) were collected at the 3 timepoints and analyzed by the Nutrition Data System for Research (University of Minnesota)
- Fasting blood samples were drawn by venipuncture into tubes containing EDTA or no anticoagulant on study day 1 and 7 (baseline), 21 and 35 (intervention), and 64 (postintervention). Not all analyses were performed on blood samples drawn on each of the 5 days.

Dependent Variables

- Clinical chemistry panels - used Beckman Synchron Lxi 725
- CBC - Cell Dyn 3200 Cell Counter
- Serum insulin concentration - immunometric assay (DPC)
- Concentrations of serum CRP - highly sensitivity immunometric assay or immunoassay kits
- Plasma concentration of nitric oxide (NO) - measurement of oxidation products, nitrite and nitrate, using the Total Nitric Oxide colorimetric assay kit
- Plasma concentrations of 42 inflammatory markers were determined using inflammatory antibody arrays III and 3.1 and optical densities with a UVP Chemi-imager (expressed as pixels of optical density) - performed on only 9 pooled plasma samples, 3 each on study day 7, 35, and 64; each plasma pool consisted of an equal volume of the plasma for the same 6 subjects on each of these study days
- Particle sizes of VLDL, LDL, and HDL, and their subfractions were analyzed using NMR techniques
- Serum concentrations of total and HDL cholesterol and triglycerides (TG) were analyzed

using automated enzymatic methods.
- LDL concentration was calculated using Friedewald equation
- Phenolic compounds were extracted from 5 g of frozen cherries and total phenolics were determined using a modified spectrophotometer Folin-Ciocalteu method. Ascorbic and dehydroascorbic acids were extracted from cherries and determined by HPLC with UV diode-array detection. Soluble solids (sugars, organic acids, amino acids, soluble pectins, phenolic, and other soluble compounds) content measured using a refractometer.

Independent Variables

- Subjects were asked to not change their activity level and diet except to replace an equivalent amount of dietary carbohydrate with carbohydrates from cherries (300 g, equivalent to 280 g when pitted at the time of consumption; ~216 kcal, ~11% daily energy intake) during the 28 days of cherry consumption.
- They were instructed to limit the consumption of foods rich in polyphenols (berries, tea, wine, grape products, fruit juice, apples, and broccoli) throughout the study.
- Bing cherries were sorted to discard cracked, decayed, and unripe fruit and weighed into aliquots of ~45 cherries each, placed in bags, and kept at 0 degrees Celsius until distribution.

Control Variables

Description of Actual Data Sample:

Initial N: 20 adults (18 males, 2 females)

Attrition (final N): 18 (2 women data excluded because one took antibiotics during the study and the other had CRP concentrations ranging from 1-31 mg/L

Age: 45 - 61 years old (mean ± SEM = 50 ± 1 years)

Ethnicity: not specified

Other relevant demographics: BMI of 20-30 kg/m^2 (mean ± SEM = 26.3 ± 0.9)

Anthropometrics

Location: University of California, Davis; USDA Western Human Nutrition Research Center

Summary of Results:

Key Findings

- After cherries were consumed for 28 days, circulating concentrations of C-reactive protein (CRP), regulated upon activation, normal T-cell expressed, and secreted (RANTES), and NO decreased by 25 ($P<.05$), 21 ($P<.05$), and 18% ($P=.07$) respectively.
- After the discontinuation of cherry consumption for 28 days, concentrations of RANTES continued to decrease ($P = .001$), whereas those of CRP and NO did not differ from either day 7 (pre-cherries) or day 35 (post-cherries).
- Plasma concentrations of IL-6 and its soluble receptor, intercellular adhesion molecule-1, and tissue inhibitor of metalloproteinases-2 did not change during the study.
- Cherry consumption did not affect the plasma concentrations of total-, HDL-, LDL-, and

VLDL-cholesterol, TG, subfractions of HDL, LDL, VLDL, and their particle sizes and numbers.
- Fasting blood glucose and insulin concentrations or a number of other chemical and hematological variables were not affected.

Other Findings

- Chemical analysis of cherries: total soluble solids were 18.3% of the fresh weight of cherries (above the minimum ripeness at harvest index of 16%) and total phenolic compounds were 134.4 mg/100 g cherries. Hydroxycinnamates were the largest class of phenolics (553.5%) followed by anthocyanins (26.4%) and procyanidins (15.8%). Only dehydroascorbic acid was detected (oxidized form of vitamin C), not ascorbic acid.
- Dietary intake: mean energy intake was 8118 kJ/day; 50.4% carbohydrate, 32.6% fat, and 16.2% protein. Saturated fat (10.5% of total daily energy intake), monounsaturated (12.3%) and polyunsaturated (7.1%). No differences pre, during, and post intervention.

Author Conclusion:

In conclusion, supplementing the diets of healthy men and women with cherries reduced the serum/plasma concentrations of some markers of inflammation, whereas circulating concentrations of many other markers of inflammation, lipids, and their subfractions, and particle sizes were not affected. The anti-inflammatory effects of cherries may be of clinical significance and should be investigated in further studies.

Reviewer Comments:

Strengths:

- *Thorough investigation of biomarkers*
- *Testing of the chemical properties of the cherries*
- *Multiple measurements*

Weaknesses:

- *No placebo group and small sample size*
- *Markers of inflammation that were reduced by cherries did not fully return to their original levels within 28 days of discontinuing the consumption of cherries (so factors other than the consumption of cherries may have contributed to the changes observed.)*
- *Supported by the California Cherry Advisory Board*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes

2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	No
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	N/A
2.3.	Were health, demographics, and other characteristics of subjects described?	No
2.4.	Were the subjects/patients a representative sample of the relevant population?	No
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	No

3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	???

6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	No
7.7.	Were the measurements conducted consistently across groups?	N/A
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	No
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes

	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		No
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	No